Figure 1

A

CIRS   X   CIRS   donor molecule with donor fragment
BS'   ▨▨▨▨   BB'

IRS   Y   IRS   receptor molecule with receptor fragment
PP'   ☐   PP'

↓ IRS recombinase
↓ *e.g.* phage ΦC31 Int protein

Y   donor molecule with receptor fragment
BP'   ☐   PB'

X   receptor molecule with donor fragment
PB'   ▨▨▨▨   BP'

B

RRS-1   X   RRS-2   donor molecule with donor fragment
◀   ▨▨▨▨   ▷

RRS-1   Y   RRS-2   receptor molecule with receptor fragment
◀   ☐   ▷

↑↓ RRS recombinase
↓ *e.g.* phage P1 Cre protein

RRS-1   Y   RRS-2   donor molecule with receptor fragment
◀   ☐   ▷

RRS-1   X   RRS-2   receptor molecule with donor fragment
◀   ▨▨▨▨   ▷

PP'	= *attP*
BB'	= *attB*
PB'	= *attR*
BP'	= *attL*
◀	= *loxP*
▷	= *lox511*

Figure 2
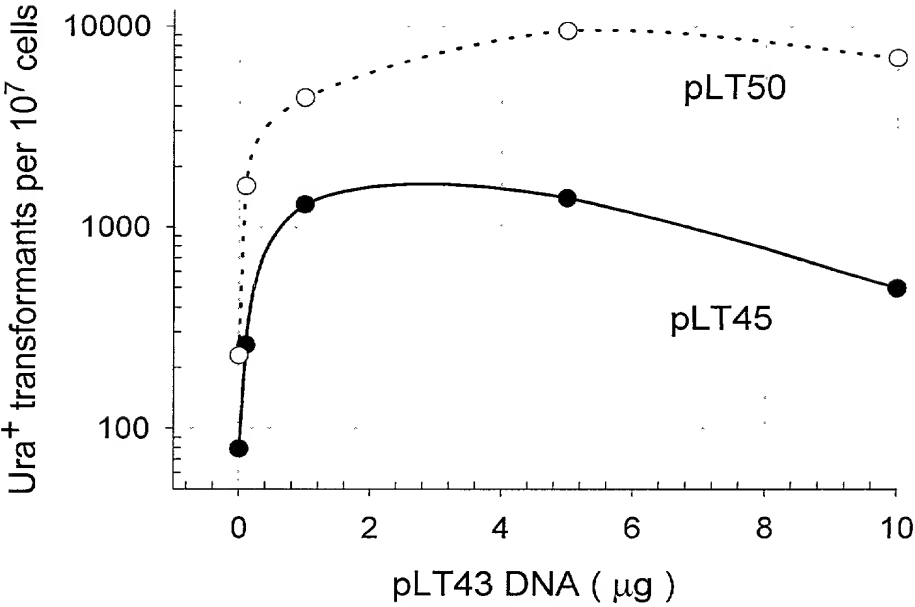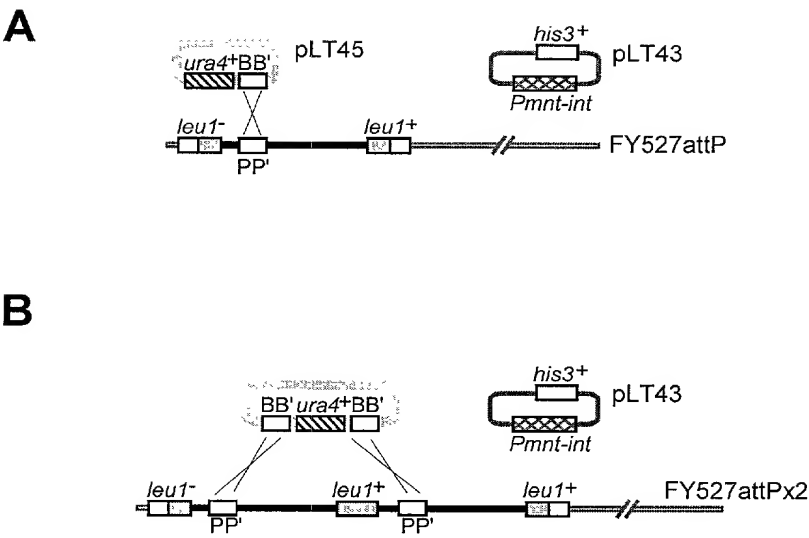
Figure 3

A

B

C

Figure 4

cDNA integration in mammalian cells

transient expression of *int*

Pc = human cytomegalovirus promoter
Ps = SV40 early promoter
zeo = zeocin resistance coding region
tk = thymidine kinase coding region
int = integrase coding region
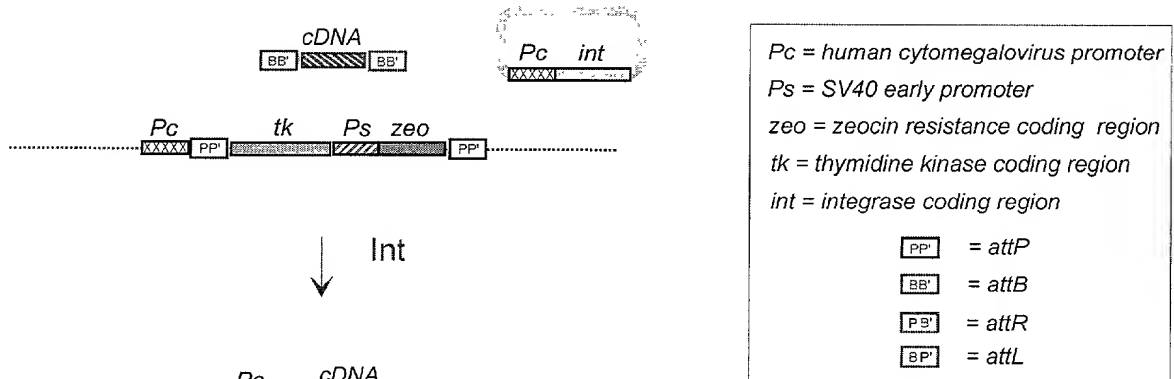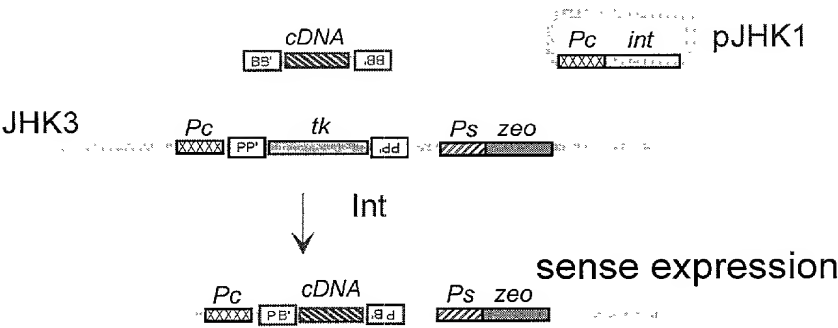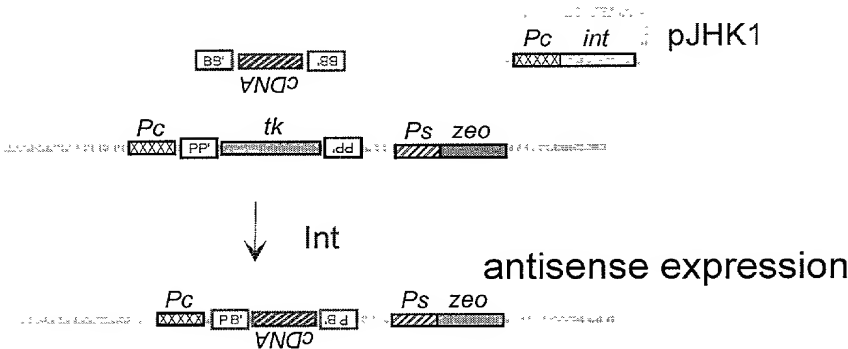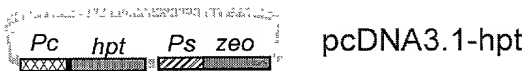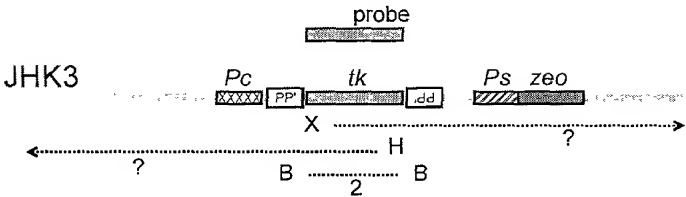
PP'	= attP
BB'	= attB
P B'	= attR
B P'	= attL

Strategy for cDNA integration in mammalian cells

A

cDNA pJHK1
BB' BB' Pc int
XXXXX

JHK3 Pc tk Ps zeo
XXXXX PP' PP' ZZZZ

Int

sense expression

Pc cDNA Ps zeo
XXXXX PB' PB' ZZZZ

B

BB' BB' pJHK1
cDNA Pc int
XXXXX

Pc tk Ps zeo
XXXXX PP' PP' ZZZZ

Int

antisense expression

Pc Ps zeo
XXXXX PB' PB' ZZZZ
cDNA

C

hpt pJHK2
BB' BB'

D

Pc hpt Ps zeo pcDNA3.1-hpt
XXXXX ZZZZ

Pc = human cytomegalovirus promoter	
Ps = SV40 early promoter	
zeo = zeocin reistance coding region	
tk = thymidine kinase coding region	
PP'	= *attP*
BB'	= *attB*
PB'	= *attR*
BP'	= *attL*

E Single copy target construct in human cells

probe

JHK3   Pc   PP'   tk   dd   Ps   zeo

X

H

B   2   B

Pc = human cytomegalovirus promoter
Ps = SV40 early promoter
zeo = zeocin reistance coding region
tk = thymidine kinase coding region

PP'	= attP
BB'	= attB
PB'	= attR
BP'	= attL

F PCR detection of DNA exchange

pJHK2

Pc   int

pJHK1

hpt
BB'   BB'

JHK3   Pc   PP'   tk   dd   Ps   zeo

Int

Pc   PB'   hpt   Bd   Ps   zeo

1.2 kb
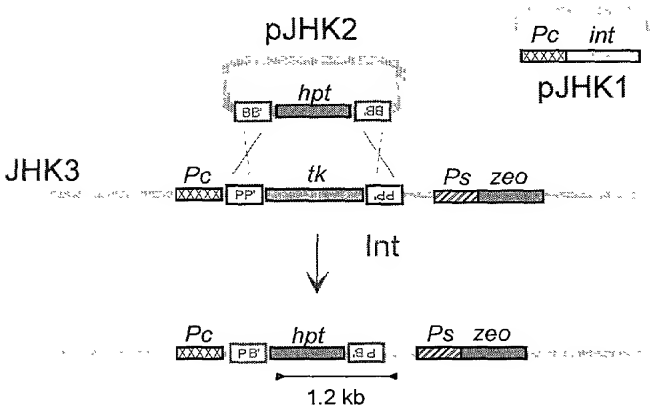
Figure 6

cDNA integration in plant cells
int expressed from target site

Figure 7

General strategy to incorporate only the trait gene
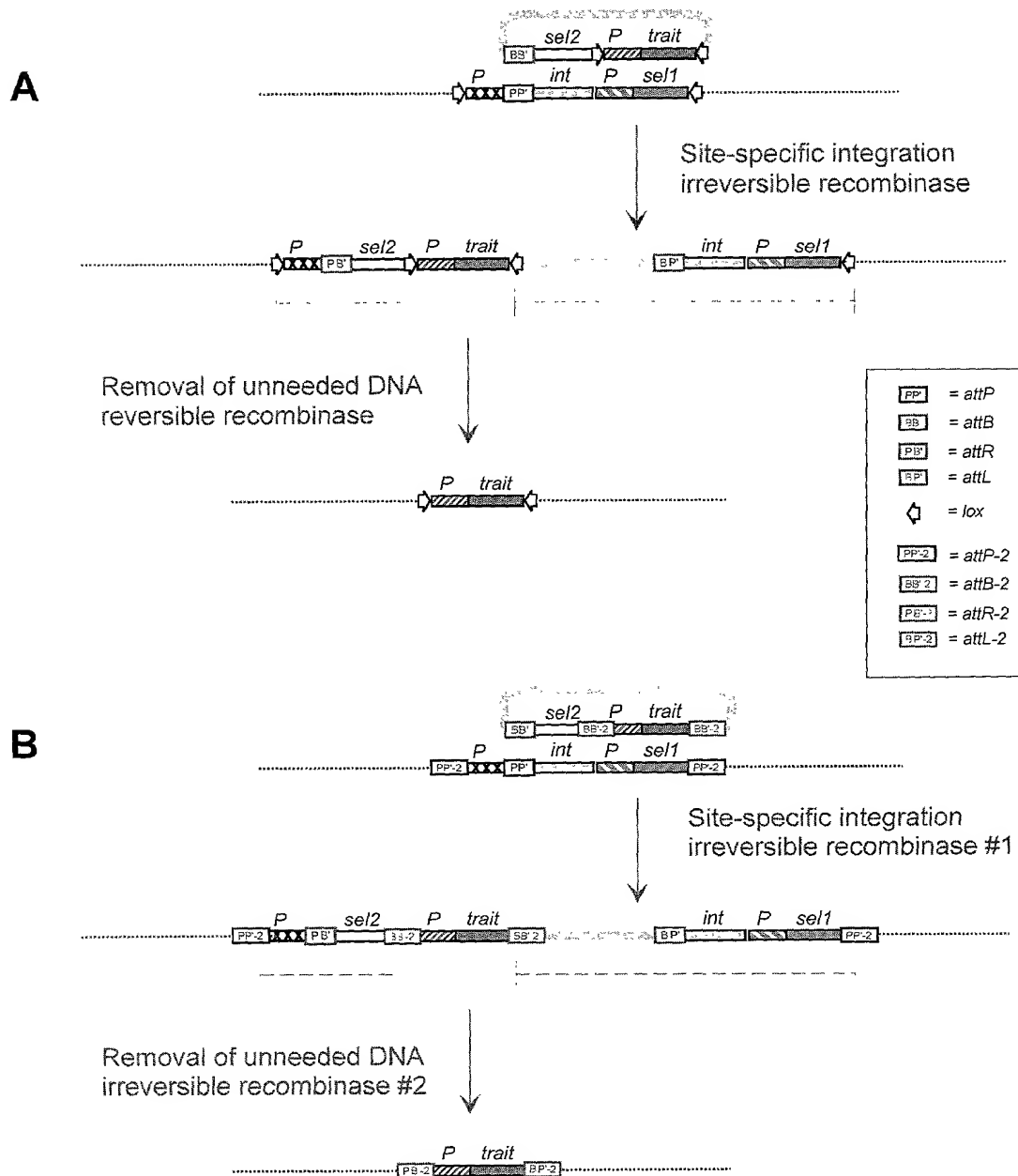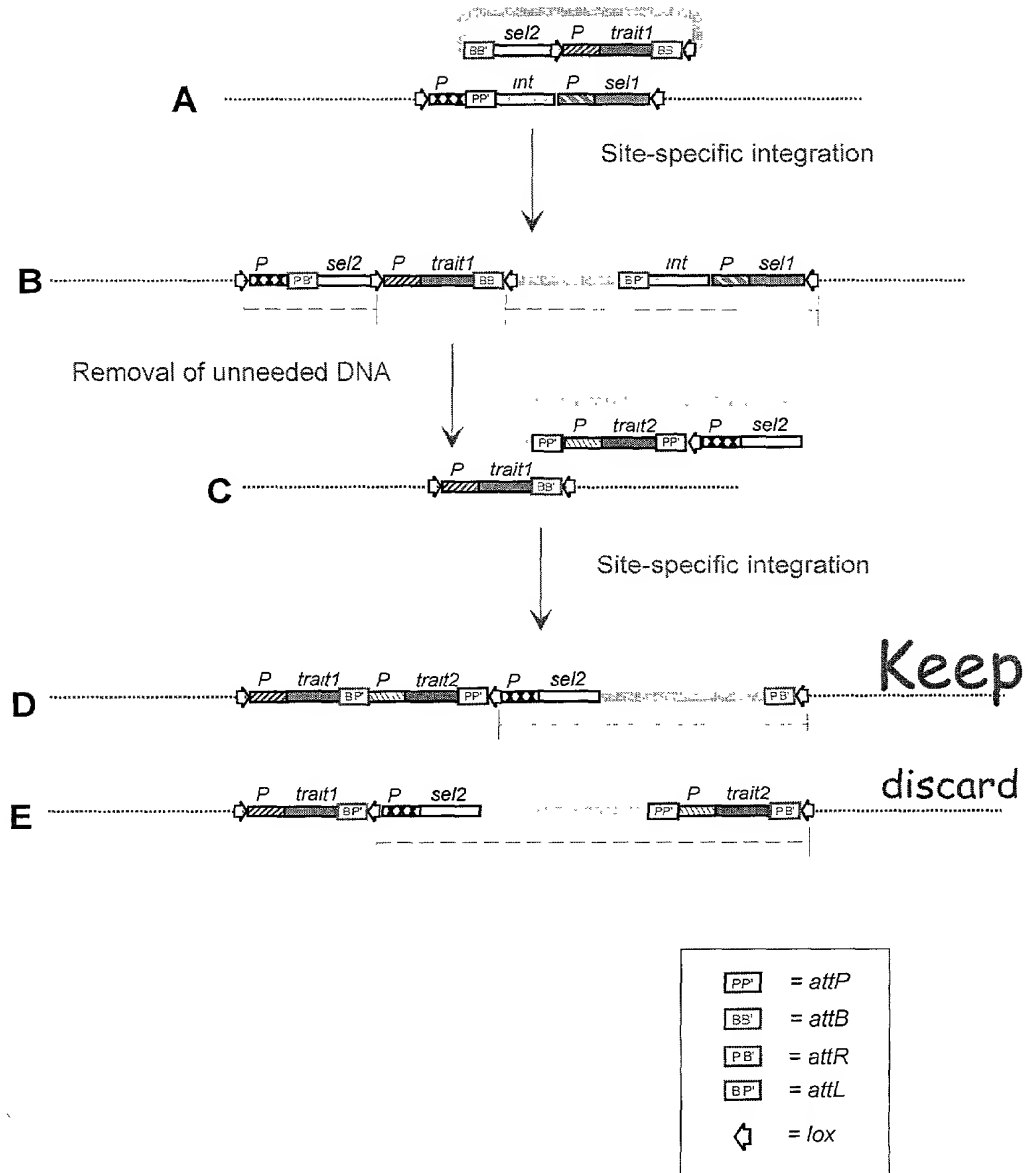
General strategy to stack genes, part1

Use of directly oriented sites

General strategy to stack genes, part2

Use of directly oriented sites

Keep

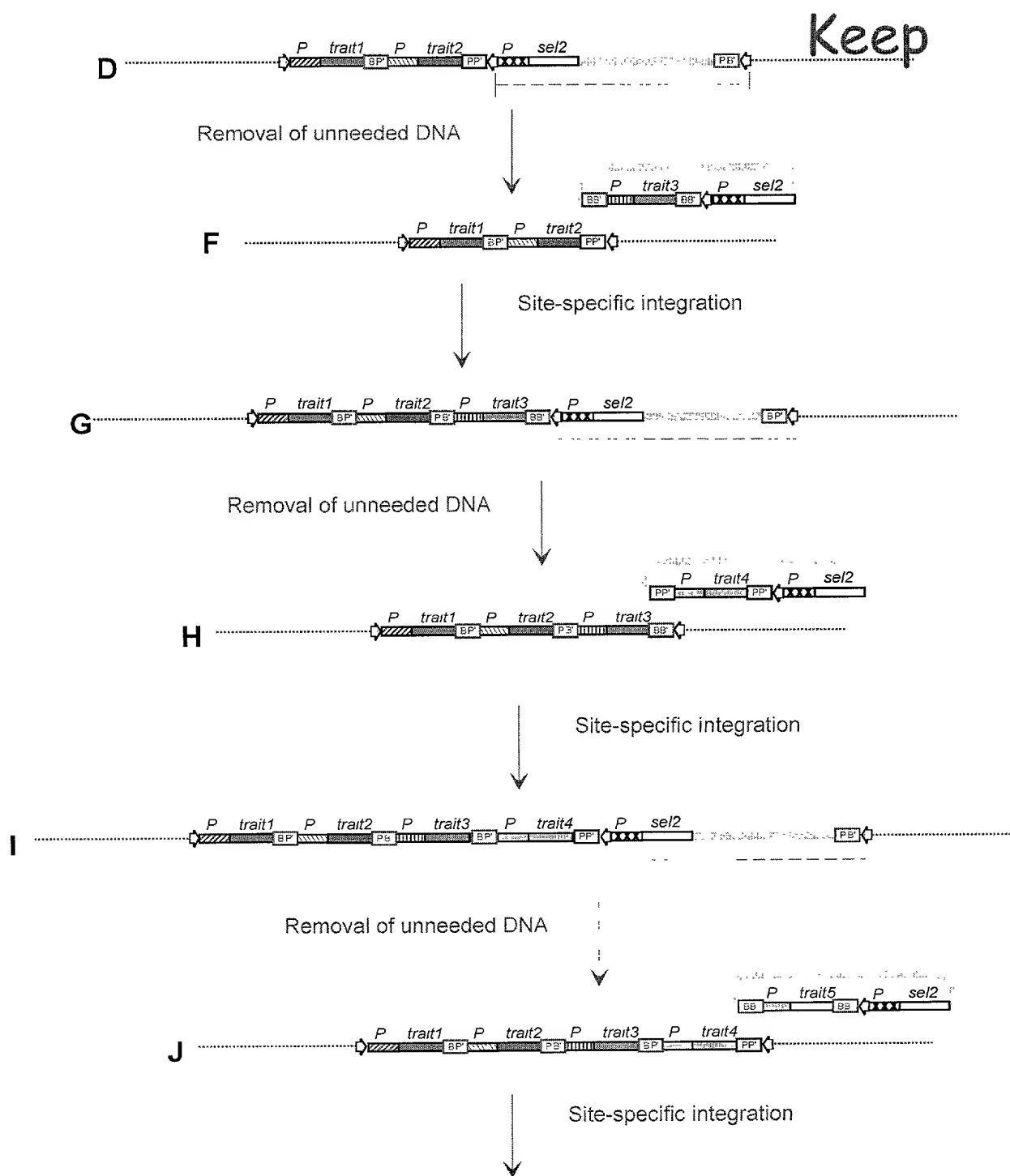General strategy to stack genes, part1

Use of inverted sites

PP'	= attP
BB'	= attB
PB'	= attR
BP'	= attL
◁	= lox
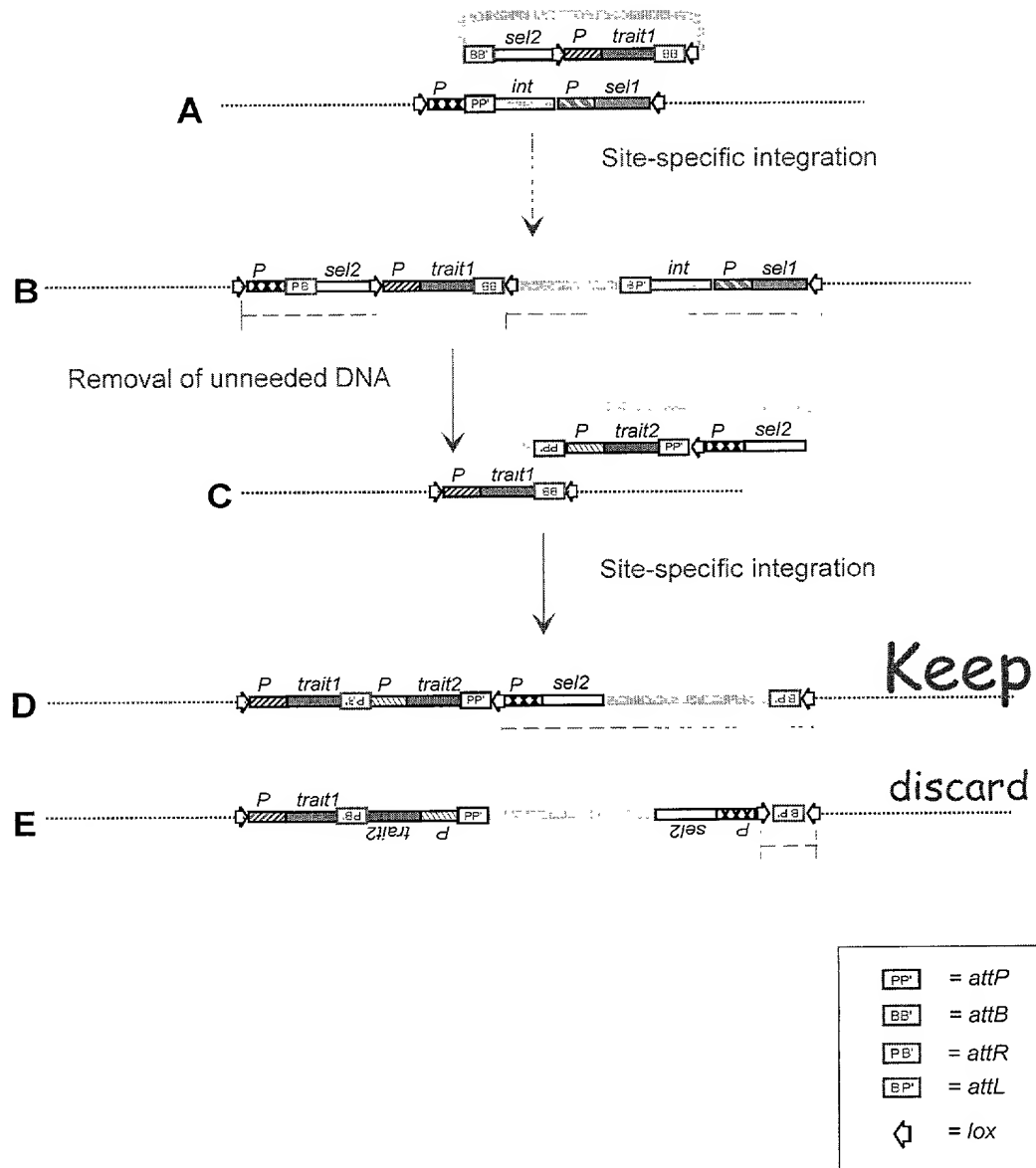
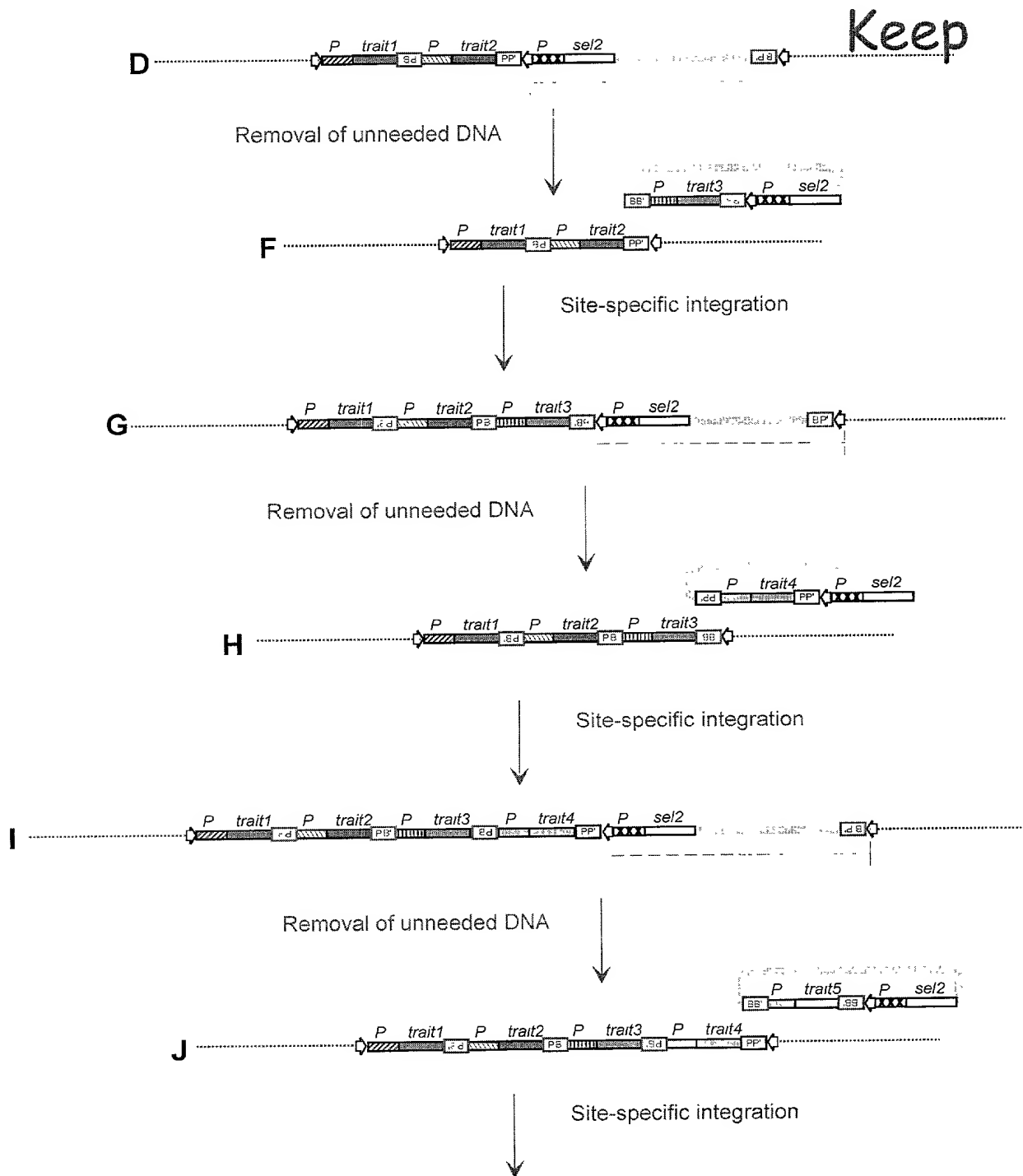General strategy to stack genes, part2

Use of inverted sites

Figure 10

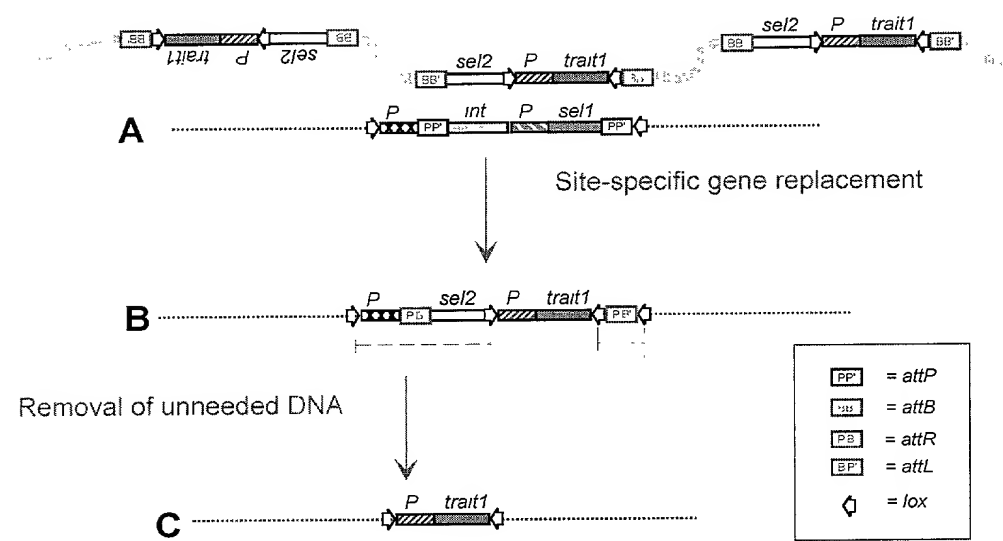Gene replacement in the host genome with directly oriented dual sites

Figure 11

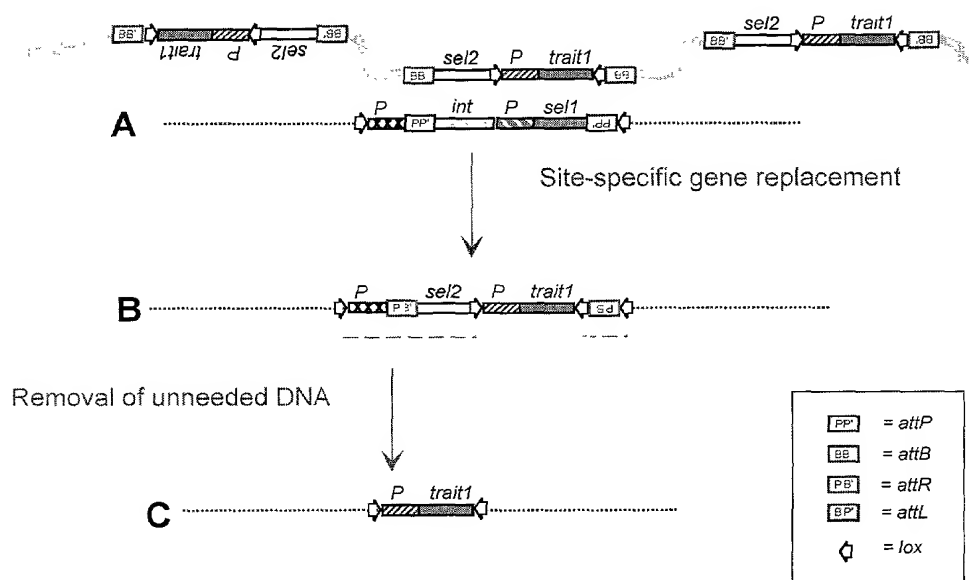Gene replacement in the host genome with inverted dual sites

Figure 12
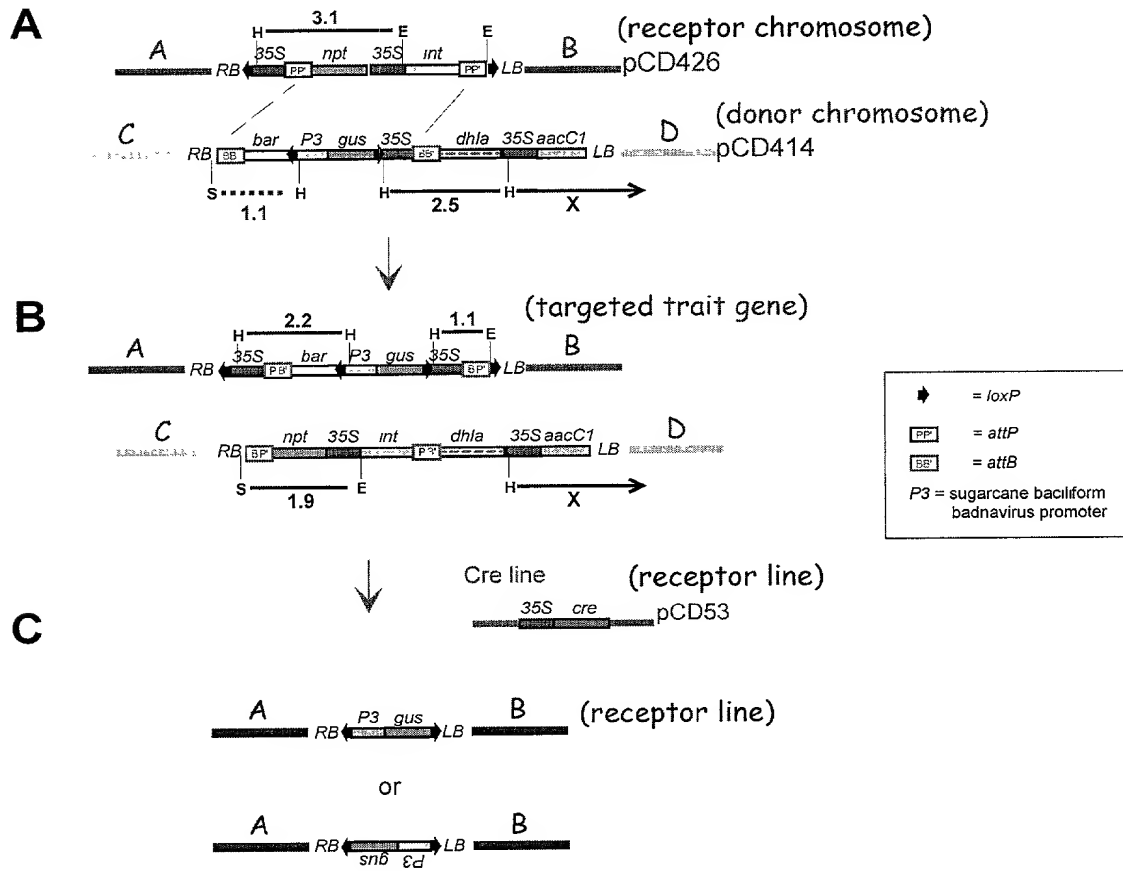
Transgene translocation from one chromosome to another

Figure 13
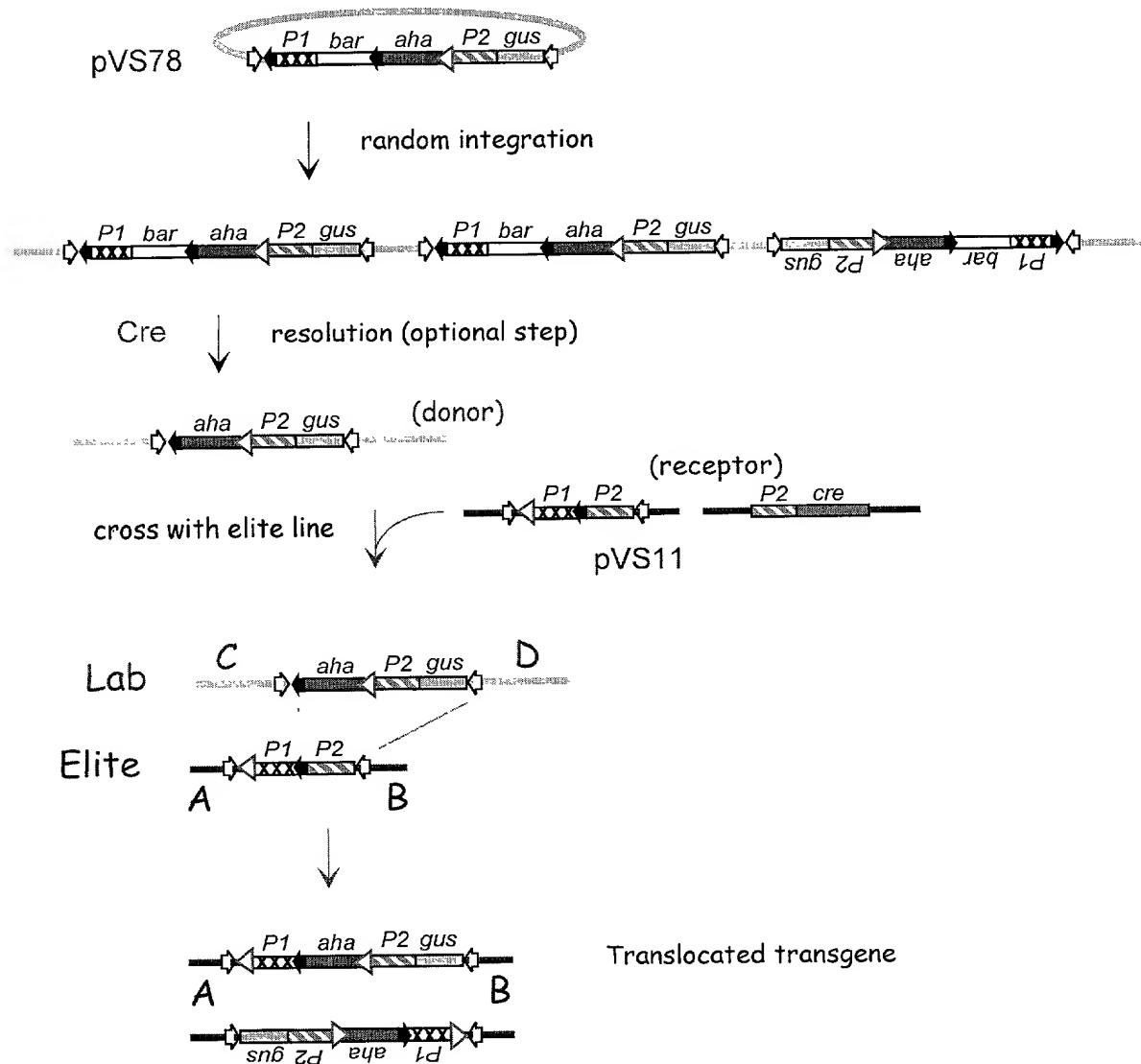
Transgene translocation using reversible recombination systems

Translocated transgene

P1 = Rice Actin promoter
P2 = Maize Ubiquitin promoter
◁ = FRT
◀ = loxP   ◁ = lox511